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TITLE: THE REGULATION OF A POST-TRANSLATIONAL PEPTIDE
         ACETYLTRANSFERASE: STRATEGIES FOR SELECTIVELY MODIFYING
         THE BIOLOGICAL ACTIVITY OF NEURAL AND ENDOCRINE PEPTIDES

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The objective of this research is to develop new strategies for pharmacologically modifyin the function of peptidergic neural and endocrine cells. The results demonstrate that chem ical agents which interact with cell surface receptors in the intermediate pituitary produ distinct changes in the molecular forms and, hence, the biological activity of β-endorphin selectively regulating the enzymes which post-translationally process the peptide. β-endor phin processing in brain is also regulated through receptor activation although, in genera it appears to be more resistant to this strategy, emphasizing the need to develop agents which control peptide processing through direct enzyme inhibition or activation. Toward t goal, experiments were initiated to isolate and characterize peptide acetyltransferase, th enzyme which N-acetylates β-endorphin and, thereby, inactivates its opiate potency. These findings emphasize the critical importance of processing enzymes in regulating peptide bic synthesis and establish the feasibility of utilizing chemical agents targeted on these en zymes to control the function of peptidergic systems. Keywords:

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FOREWORD

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INTRODUCTION

The goal of this research program is to develop new approaches for modifying the function of peptidergic systems by using chemical agents to induce selective changes in the activity of the enzymes which post-translationally process neural and endocrine peptides. Post-translational processing enzymes are essential components of peptide biosynthetic pathways because most neuropeptides are initially synthesized in the form of a larger, biologically inactive precursor molecule. Peptide precursors are then enzymatically cleaved to smaller, bioactive peptides. These commonly undergo additional, non-proteolytic enzymatic modifications which further define their activity. Thus, post-translational processing enzymes play a critical role in determining the biological activity of neural and endocrine peptides. This research is, therefore, predicated on the hypothesis that alterations in the activity of peptide processing enzymes, whether induced directly by specific enzyme inhibitors or indirectly, by agents which act through cell surface receptors, will lead to selective and predictable changes in the physiological responses mediated by peptidergic neural and endocrine systems.

When we initiated these studies little was known about the regulation of post-translational processing enzymes or of the role of enzyme regulation in controlling peptide biosynthesis. The first phase of our studies, therefore, had two objectives; first, to address the basic question; are post-translational processing enzymes regulated? Specifically, do they undergo classical enzyme induction following treatments which accelerate the rate of synthesis of their substrates? Secondly, we sought to determine whether the post-translational processing and, hence, the biological activity, of neuroendocrine peptides can be modified by agents which alter the biosynthetic activity of peptidergic cells by acting on cell surface receptors.

To address these questions we focused our research on the β-endorphin processing pathway in the intermediate lobe of the rat pituitary. The intermediate lobe is an ideal model system because it is a homogeneous tissue, 98% of its cells synthesize high levels of β-endorphin and other peptide derivatives of pro-opiomelanocortin (POMC) (1), and because it is controlled by a relatively uncomplicated neuronal innervation; inhibitory dopaminergic neurons projecting from the hypothalamus regulate secretion from the gland (2). As a result, dopamine receptor agonists inhibit and antagonists stimulate the biosynthesis and release of β-endorphin (3-6). This provides us with an experimental paradigm with which we can pharmacologically manipulate POMC biosynthesis and then examine the regulation of β-endorphin processing.

Earlier studies had shown that β-endorphin is initially synthesized as a thirty-one amino acid peptide which is then further processed (Fig. 1). β-endorphin-1-31 is first N-acetylated by a specific post-translational peptide acetyltransferase and then it undergoes sequential C-terminal proteolysis to from N-acetyl-β-endorphin-1-27 and -1-26 (7). The endoproteolytic cleavage of β-endorphin-1-31 occurs at the C-terminal of a pair of lysine residues which are then removed by a carboxypeptidase B-like enzyme, carboxypeptidase H, which characteristically removes lysine and arginine residues remaining after the

endoproteolytic cleavage of peptides. Small amounts of non-acetylated β-endorphin-1-27 and -1-26 are also formed through a second, quantitatively minor pathway. Only two of the enzymes which process β-endorphin have been firmly identified, peptide acetyltransferase and carboxypeptidase H. However, carboxypeptidase H is also thought to remove the C-terminal histidine from β-endorphin-1-27, forming β-endorphine-1-26 (7).

The β-endorphin processing pathway is especially intriguing for two reasons. First, the processing of β-endorphin substantially modifies its biological activity. Both N-acetylation and C-terminal cleavage essentially abolish the opioid activity of β-endorphin-1-31 (8). Moreover, C-terminal proteolysis also produces a potent opioid receptor antagonist, β-endorphin-1-27 (9). β-endorphin-1-26, on the other hand, is devoid of both opioid agonist and antagonist activity. (8,9). This means that most β-endorphin neurons and endocrine cells release opiate active, antagonist and inactive forms of the peptide and emphasizes the feasibility of selectively altering the physiologic effects of β-endorphin with chemical agents targeted on processing enzymes. The second interesting feature of the β-endorphin processing pathway is that it is heterogeneous. This means that the intermediate lobe releases not only the final product of the processing pathway, N-acetyl-β-endorphin-1-26, but also substantial amounts of the precursor and intermediates (7, 10). This suggests that the regulation of β-endorphin processing enzymes is critical to the expression of the multiple forms of β-endorphin released from the intermediate lobe.

Our initial, Phase I experiments were largely completed during the first year of the project. They clearly demonstrated that at least one processing enzyme, peptide acetyltransferase, is regulated; it undergoes classical enzyme induction following treatments which accelerate the firing frequency of melanotrophic cells (6). Our first experiments were designed to characterize the effects of chronic treatment with haloperidol, a dopamine receptor antagonist, and bromocriptine, a dopaminergic agonist, on the biosynthesis, content and release of β-endorphin. We found that chronic haloperidol treatment produced sustained increases in POMC mRNA levels and in the content and release of β-endorphin and that bromocriptine lowered POMC mRNA and peptide levels (6). Time course and dose response studies further demonstrated that peptide acetyltransferase activity co-varied with POMC biosynthesis and kinetic analyses indicated that peptide acetyltransferase is regulated through changes in the amount of the enzyme in the intermediate lobe and not by alterations in its substrate affinity. Together, these experiments indicate that peptide acetyltransferase activity is co-regulated with the rate of POMC biosynthesis, presumably through the coordinated transcriptional regulation of the genes encoding the two proteins. This appears to be the only mechanism for regulating the enzyme and we have found no evidence that it is regulated by short term changes in its substrate affinity.

We next examined the effects of haloperidol and bromocriptine treatments on the post-translational processing of β-endorphin. This revealed that the acetylation of β-endorphin peptides was not affected by either treatment, suggesting that the induction of peptide acetyltransferase functions as a mechanism for maintaining the acetylation of β-endorphin peptides independent of changes in the synthesis and release of the peptide. We also found, however, that

the C-terminal proteolysis of β-endorphin was differentially altered by both haloperidol and bromocriptine. Specifically, haloperidol treatment reduced and bromocriptine facilitated the conversion of N-acetyl-β-endorphin-1-27 to -1-26, suggesting that the enzyme which catalyzes this step, which is thought to be carboxypeptidase H, is not regulated. Consistent with this hypothesis, we found that carboxypeptidase H activity was not induced by haloperidol. This demonstrates that carboxypeptidase H, unlike peptide acetyltransferase, is not an inducible enzyme.

These results indicate that post-translational processing enzymes are regulated through two distinct mechanisms. In one case, peptide acetyltransferase, the enzyme is present in low levels, it is an inducible enzyme and the processing step it catalyzes is heterogeneous; both the substrate and product of the enzymatic reaction are present. In a second case, exemplified by carboxypeptidase H, the enzyme is present in high levels (approximately 1000 times higher than peptide acetyltransferase) it is not inducible and its primary substrates, peptides with C-terminal lysine and arginine residues remaining after the endoproteolytic cleavage of peptide precursors, are completely absent; they are entirely converted to products. However, carboxypeptidase H apparently functions differently in its second role, converting β-endorphin-1-27 to -1-26 because this is a heterogeneous processing step; both β-endorphin-1-27 and -1-26 are present in the intermediate lobe in their acetylated forms. Based on preliminary results, this apparently occurs because the affinity of carboxypeptidase H for the C-terminal histidine of β-endorphin-1-27 is much lower, three to four orders of magnitude lower, than its affinity for arginine and lysine residues. Consequently, in this case, the enzyme functions as a heterogeneous processing enzyme as a result of its lower affinity for histidine.

These experiments revealed two new facts about peptide processing. First, they conclusively demonstrate that post-translational processing enzymes are selectively regulated. On the one hand, peptide acetyltransferase is induced by treatments which accelerate POMC biosynthesis but, on the other, carboxypeptidase H is not similarly regulated. Secondly, they show that the post-translational processing of β-endorphin is not rididly controlled but rather that it can be modified by changes in the biosynthetic activity of melanotropic cells. Together, these results clearly indicate that post-translational processing enzymes are rate limiting steps in peptide biosynthesis. This means that chemical agents designed to inhibit or activate these enzymes will have profound effects on the peptides they synthesize. Furthermore, these studies establish the feasibility of utilizing chemical agents targeted on cell surface receptors to selectively alter the post-translational processing and, thus, the biological activity of neural and endocrine peptides.

These studies also led to several quite intriguing, yet unexpected findings, which demonstrate that the response of the intermediate pituitary to dopaminergic agents is considerably more dynamic and multifaceted than we anticipated, based on existing knowledge of pituitary function. These results, which will be summarized in the results section of the present report, essentially conclude Phase I studies of the coordinate regulation of peptide acetyltransferase, POMC gene expression and β-endorphin processing in the intermediate pituitary.

Phase II of this research project concerns itself with the presence and regulation of peptide acetyltransferase and other β-endorphin processing in brain. These studies are an essential next step toward our ultimate goal of developing strategies, targeted on processing enzymes, for modifying the physiological and behavioral responses mediated by β-endorphin and other peptides in brain. They address three interrelated questions. First, are the same β-endorphin processing enzymes that we identified in the intermediate lobe also expressed in brain. We tested this during the first year of the project by measuring the precursors and products of these enzymes, the molecular forms of β-endorphin. We found that all of the forms of the peptide present in the intermediate lobe are also found in brain although their relative proportions varied among brain regions. However, acetylated forms were quantitatively minor. This implied that perhaps peptide acetyltransferase is not a critical determinant of the bioactivity of β-endorphin in brain. If so, then chemical agents targeted on this enzyme may be of limited clinical utility and our efforts might be better spent studying the enzymes which C-terminally cleave β-endorphin. To further address this question, we examined the molecular forms of β-endorphin in human brain. Our initial results indicate, however, that, in the human, peptide acetyltransferase plays a major role in processing β-endorphin. Furthermore, recent studies have also shown that acetylated β-endorphin peptides predominate in the caudal medulla of the rat where β-endorphin neurons play an important role in regulating the cardiovascular system (12). These observations indicate that peptide acetyltransferase does, indeed, play a key role in controlling the physiology of brain β-endorphin.

The second question addressed by Phase II studies is whether β-endorphin processing is regulated in the same manner in brain as in the pituitary; these studies have been completed during the current project period. They demonstrated that the post-translational processing of β-endorphin in brain can be altered by chemical agents which interact with cell surface receptors although, in general, brain β-endorphin processing appears to be more resistant to modification by this strategy than the intermediate pituitary. This emphasizes the need to develop agents which will alter peptide acetyltransferase activity by directly inhibiting or activating the enzyme.

The third question raised by Phase II studies concerns the ultimate consequences of chemically induced changes in β-endorphin processing. To predict this it is first necessary to determine the effects of the differing molecular forms of the peptide which are actually present in brain. We recently initiated studies of the effects of these peptides on two physiologic parameters which are among the most conclusively identified roles of the peptide; analgesia and cardiovascular regulation. These results remain preliminary in nature and will not be included in the present report.

Phase III of this project will focus on the isolation and characterization of peptide acetyltransferase. Phase II studies established the need for developing agents which will act directly on this and, ultimately, on other β-endorphin processing enzymes in order to modify the biological activity of β-endorphin in brain. To accomplish this objective it will be necessary to purify peptide acety transferase in order to test enzyme inhibitors and to determine the structure activity relationships of the enzyme. Methods for testing these inhibitors in vivo and/or in tissue culture are also necessary because inhibitors that are

effective in test tube assays may not be effective in vivo if they do not cross biological membranes. Experiments have been initiated during the curreng project period to develop these methods and to begin to isolate peptide acetyltransferase.

In summary then, the present report will first review the final chapter of Phase I studies of the co-regulation of peptide acetyltransferase, POMC biosynthesis and β-endorphin processing in the endocrine POMC system. These experiments have provided us with a more difinitive understanding of the role of processing enzyme regulation in the overall biochemical and cellular response of the intermediate lobe to environmental stimuli. Phase II studies of the presence and regulation of β-endorphin processing enzymes in brain, which are largely complete, yet still ongoing, will also be discussed with particular emphasis on our recent investigations of the post-translational processing of β-endorphin in human brain. Finally, our initial progress toward the isolation and characterization of peptide acetyltransferase, the third and final phase of the project, will be summarized.

EXPERIMENTAL RESULTS

1. The co-regulation of peptide acetyltransferase and POMC biosynthesis: Dopamine receptor activation produces coordinated changes in POMC gene expression and the subcellular morphology of the intermediate pituitary.

a) Dopaminergic regulation of the biosynthetic activity of individual intermediate lobe cells: Evaluation by in situ hybridization and morphometric analysis.

Chronwall, B.M., Hook, G.R. and Millington, W.R. Dopaminergic regulation of the biosynthetic activity of individual melanotrophs in the rat pituitary intermediate lobe: A morphometric analysis by light and electron microscopy and in situ hybridization. (Submitted to Endocrinology).

The initial objective of these studies was to evaluate haloperidol- and bromocriptine induced changes in POMC gene expression using in situ hybridization, a technique in which POMC mRNA is detected by hybridizing a cDNA POMC probe directly on tissue sections, thereby quantifying changes in POMC mRNA within individual cells of the intermediate pituitary (13). This would enable us to investigate whether coordinate changes in POMC gene expression and peptide acetyltransferase activity were cell specific or if they occurred equally in all intermediate lobe cells. The results confirm our earlier experiments with 'dot-blot' hybridization assays which demonstrated that long term haloperidol treatment elevates and bromocriptine reduces POMC mRNA levels in all cells of the intermediate lobe (6). However, these studies produced several additional, unexpected findings showing that dopaminergic agents induce a coordinated set of biochemical and cellular responses which are manifested according to a distinct temporal pattern following acute, subchronic and chronic treatment.

The initial, unexpected result of these studies was that dopaminergic agents produce rapid changes in POMC mRNA levels. Haloperidol (2 mg/kg) elevated POMC mRNA levels not only after chronic administration, as we had previously shown (6), but also after acute (6 h) and subchronic treatments. Detailed time course studies further demonstrated that POMC mRNA was elevated within four hours of a single haloperidol injection (5 mg/kg) and remained above control values for twenty-four hours; after a lower dose (2 mg/kg) it was elevated only transiently. This indicates that acute blockade of dopamine receptors produces rapid changes in POMC gene transcription, perhaps within minutes of treatment.

The second finding revealed by in situ hybridization was that POMC mRNA is not uniformly distributed among intermediate lobe cells as we had expected. POMC mRNA levels differed among individual cells of the gland, suggesting that the intermediate lobe contains two classes of cells maintaining differing rates of POMC biosynthesis. Subchronic haloperidol treatment not only increased the amount of POMC mRNA in each cell but also eliminated the hetereogeneity in its distribution. Bromocriptine lowered POMC mRNA levels and also reduced this cellular heterogeneity. This further indicates that the heterogeneous distribution of POMC mRNA was not an artifact resulting from differences in tissue thickness or other experimental variables.

To further test the concept that the intermediate lobe cells synthesize POMC at different rates, we conducted morphometric studies using both light and electron microscopy. Both approaches showed that intermediate lobe melano-trophs differ in the tinctorial properties of their cytoplasm; some cells appeared distincly darker, others lighter. Morphometric studies showed why this is so. Darkly staining cells have a denser cytosol and contain a significantly greater amount of the subcellular organelles which synthesize and store secretory proteins. The amount of rough endoplasmic reticulum, Golgi, secretory vesicles and mitochondria were all higher in dark cells than in light. These results support the conclusion that dark cells are engaged in a high level of POMC biosynthesis while light cells are biosynthetically quiescent, normally producing relatively little POMC. Subchronic haloperidol administration converted all of the light cells into darkly staining ones which, on morphometric analysis, exhibited a high density of protein synthesizing organelles. Thus, subchronic treatment had two effects on the intermediate pituitary, both of which independently increased the total production of POMC in the gland; it increased POMC mRNA levels in all cells and it stimulated biosynthetically quiescent light cells to produce high levels of POMC. Subchronic bromocriptine had the opposite effects on these two parameters. Together, these data suggest that light cells represent a reserve biosynthetic capacity of the intermediate lobe, normally 'on idle' they can be 'reved up' during periods of high demand.

We have also shown that longer term treatment (12-21 days) with dopaminergic agents produced yet another cellular response in the intermediate pituitary; chronic haloperidol stimulated and bromocriptine reduced the rate of cell proliferation in the gland (13). This first became apparent when, during in situ hybridization experiments, we noticed that haloperidol treatment increased the number of cell layers in the intermediate lobe without affecting the size of

individual cells. We then utilized (^{3}H)-thymidine uptake experiments and quantitation of the mitotic index to confirm that this hyperplastic response resulted from an increase in cell proliferation and not from selective cell death (13).

These data show that activation or inhibition of cell surface receptors produces a much more complex and coordinated panoply of biochemical and cellular responses than previously anticipated. Acute haloperidol treatment stimulates rapid changes in POMC gene transcription and, hence, POMC biosynthesis. Subchronic treatment continues to accelerate POMC biosynthesis and also converts light cells, the biosynthetic reserve of the intermediate lobe, into dark cells, those actively engaged in syntheizing POMC, thus further expanding the biosynthetic capacity of the gland as a whole. Finally, after prolonged haloperidol treatment, the intermediate lobe exhibits cellular hyperplasia, an increase in the number of cells in the gland. Thus, the intermediate lobe has three independent, yet coordinated, mechanisms for modulating its peptide output. Only after chronic treatment, however, do we observe selective changes in the activity of post-translational processing enzymes and concomitant alterations in β-endorphin processing. Thus, alterations in β-endorphin processing appear to be a signal to the periphery that the organism is undergoing a persistent physiological stimuli, perceived by the central nervous system and communicated through the intermediate lobe.

b) Equine Cushing's syndrome: A model for prolonged changes in the dopaminergic regulation of the intermediate pituitary: The experimental results described above prompted us to consider whether the coordinated regulation of peptide acetyltransferase and POMC gene expression would persist if we continued the chronic administration of dopaminergic agents for longer periods of time. Unfortunately, treating rats for such prolonged periods is impractical. However, we have identified a spontaneous condition, a hyperplasia of the intermediate pituitary which occurs in the horse, producing a Cushing's-like syndrome (14), which is thought to result from a central denervation of the intermediate lobe, similar to the chemical denervation induced by chronic haloperidol.

Our initial experiments were designed to test the hypothesis that the development of intermediate lobe hyperplasia in the horse was, indeed, associated with a loss of dopaminergic innervation. To do so, we measured the concentrations of dopamine, serotonin and their respective metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) by high performance liquid chromatography (15). We found that dopamine levels were reduced by a factor of ten in intermediate lobe tissue from Cushing's horses and that DOPAC levels were completely undetectable (Table 1). Interestingly, levels of serotonin and 5-HIAA were not significantly different from control animals. These results show that, in equine Cushing's syndrome, the dopaminergic innervation of the intermediate lobe is almost completely lost while serotonergic neurons apparently remain intact. Thus, as in the case of the 'chemical denervation' produced by haloperidol, equine Cushing's syndrome is associated with a relatively specific loss of the dopaminergic regulation of the intermediate lobe.

Subsequent experiments showed that the intermediate lobe hyperplasia of equine Cushing's syndrome produces profound elevations in both plasma and cerebrospinal fluid (CSF) β-endorphin levels; β-endorphin was increased 60- and 120-fold in plasma and CSF, respectively (Table 2). Nevertheless, there were no changes in either β-endorphin or POMC mRNA levels within the intermediate lobe itself (Table 3). This indicates that, while the number of cells in the gland is substantially increased, the rate of POMC biosynthesis in individual intermediate lobe cells is unchanged.

We also found that the post-translational processing of β-endorphin was significantly altered in equine Cushing's syndrome relative to age matched controls (Table 4; Fig. 2). There was proportionately more β-endorphin-1-31 and lower levels of N-acetylated and C-terminally shortened forms of the peptide. This response is distinctly different from that observed following chronic haloperidol treatment although we have found comparable changes in β-endorphin processing in the absence of altered biosynthesis in rat brain following chronic haloperidol. This finding suggests either that β-endorphin processing enzymes are reduced in activity in equine Cushing's syndrome or that the duration of time between synthesis and secretion is reduced, leading to incomplete processing of the peptide. In either case, the data lend firm support to the concept that alterations in β-endorphin processing do occur in the absence of parallel changes in POMC biosynthesis. Moreover, these results indicate that, after prolonged alterations in the dopaminergic regulation of the intermediate lobe, cellular hyperplasia alone accounts for the increased peptide output of the gland; POMC gene transcription and the discrimination of light and dark cells has returned to control levels.

These experiments also revealed that anterior pituitary corticotrophs of the horse contain acetylated β-endorphin peptides, unlike the anterior lobe of the rat and every other mammalian species thus far examined. Cation exchange chromatography showed that acetylated forms of β-endorphin-1-31, -1-27 and -1-26 constituted approximately 35% of total β-endorphin in the anterior lobe. Combined immunocytochemistry and in situ hybridizatior studies confirmed the presence of acetylated β-endorphin in anterior lobe corticotrophs. These data show that critical species differences occur in the distribution of peptide acetyltransferase and may potentially be useful in determining the factors which control the tissue specific expression of the peptide acetyltransferase gene as well as the functional role subserved by the tissue, and species, specific acetylation of β-endorphin peptides.

2. The regulation of β-endorphin processing in rat brain.

Berglund, L.A., Millington, W.R. and Simpkins, J.W. Gonadal steroid and chronic morphine treatments do not change the post-translational processing of β-endorphin in rat brain. (Submitted to Life Sciences).

During the initial year of this research project we examined the effect of chronic haloperidol treatment on the post-translational processing of β-endorphin in rat brain. This showed that β-endorphin processing was altered by haloperidol treatment in the midbrain; there was a higher proportion of β-endorphin-1-31 and lower levels of N-acetylated and C-terminally shortened forms.

Interestingly, these changes were not accompanied by elevated β-endorphin levels. This indicates that haloperidol altered β-endorphin processing without affecting POMC biosynthesis, which clearly differs from the regulation of β-endorphin processing in the intermediate pituitary. During the current project period we continued studies of the regulation of brain β-endorphin processing, examining the effects of two treatment paradigms with known effects on POMC biosynthesis; gonadal steroid treatment and chronic morphine.

Estradiol treatment of ovariectomized rats lowers hypothalmic POMC mRNA (16) and decreases β-endorphin levels in the hypothalamus and brainstem (17, 18); progesterone treatment antagonizes this response (17). These effects are of physiologic importance because the sequential administration of estradiol and progesterone to ovariectomized rats triggers the LH surge, a response thought to be mediated by a transient reduction in the tonic inhibitory control of LH release maintained by hypothalamic β-endorphin neurons (19). The LH surge is also associated with a refractoriness to exogenous opiates, which indicates that the inhibition of β-endorphin synthesis and release does not fully account for the response. We hypothesized that this desensitization could result from a steroid induced change in the enzymatic processing of β-endorphin, causing β-endorphin neurons to release a higher proportion of the opiate antagonist, β-endorphin-1-27, and less of the opiate agonist, β-endorphin-1-31. This was an ideal paradigm for examining the regulation of β-endorphin processing because a well characterized physiologic output, the LH surge, could be correlated with changes in processing. Furthermore, clear evidence was available that gonadal steroids lower the rate of POMC biosynthesis and, in the intermediate pituitary, we knew that alterations in POMC synthesis were associated with distinct changes in the post-translational processing of β-endorphin.

We found, however, that gonadal steroid treatment had no effect on the post-translational processing of β-endorphin in rat brain. There were no significant differences in β-endorphin processing between controls and rats treated with either estradiol alone or estradiol plus progesterone in either the preoptic area of the hypothalamus, the medial basal hypothalamus or the brainstem. Thus, the LH surge is not associated with altered β-endorphin processing. Moreover, these results demonstrate that changes in POMC biosynthesis in brain, unlike the intermediate pituitary, do not produce parallel changes in β-endorphin processing.

We have also examined the effect of chronic morphine administration on the post-translational processing of β-endorphin in rat brain. Chronic morphine renders rats, as well as humans, tolerant and dependent to the drug. Thus, any morphine induced changes in β-endorphin processing, that is, in the ratio of opiate agonist and antagonist forms of the peptide, could have important implications for the mechanism of opiate dependence. Earlier reports had shown that chronic morphine lowers both β-endorphin (20) and POMC mRNA levels (21) in rat brain and reduces the rate of β-endorphin release into the hypophysial portal vasculature, strong evidence that morphine inhibits the synthesis and release of hypothalamic β-endorphin (22).

Morphine dependence was induced in rats by implanting sustained release morphine pellets (75 mg/pellet) subcutaneously, one pellet on the first day of the experiment and two additional pellets on day three; the animals were killed on day five. We found, however, that morphine dependence did not produce any changes in the post-translational processing of β-endorphin in either the hypothalamus or the periaqueductal grey, a region associated with the analgetic effect of both morphine and β-endorphin. Again, these results indicate that treatments which alter the rate of POMC biosynthesis in brain do not affect the post-translational processing of β-endorphin.

To summarize, we have examined the effects of three different treatment paradigms on β-endorphin processing in rat brain; chronic haloperidol, gonadal steroids and morphine tolerance and dependence. Only the first, haloperidol treatment, affected processing but this was not associated with altered POMC biosynthesis. Gonadal steroid and morphine treatments, which do alter POMC synthesis, do not affect β-endorphin processing. These results indicate that β-endorphin processing in brain is regulated differently than in the inter-mediate pituitary and is not affected by treatments which alter POMC synthesis. Additional, yet to be identified mechanisms appear to regulated brain β-endorphin processing.

3. The post-translational processing of β-endorphin in human brain.
The presence of β-endorphin immunoreactivity in human brain was first demonstrated soon after the peptide was first identified in the mid 1970s. Its distribution is similar to that of the rat and other mammals (23,24) and, like the rat, most of the immunoreactive β-endorphin in human brain is localized within neuronal processes which arise from cell bodies in the medial basal hypothalamus (25). However, exactly how β-endorphin is processed in the human, whether to opiate active or inactive forms, is completely unknown. This information is essential to our long-term goal of developing clinically useful pharmacologic strategies for modifying the processing of β-endorphin in brain. We, therefore, initiated studies of the post-translational processing of β-endorphin in human hypothalamus and CSF during this project period. These experiments indicate that all of the β-endorphin forms which we identified in rat brain are also expressed in the human although, unlike the rat, acetylated β-endorphin peptides are quantitatively major forms of the β-endorphin processing pathway in human brain.

Prior to initiating these studies we addressed a problem common to studies which utilize human autopsy material; the effect of the postmortem interval, that is, the duration of time between death and freezing the tissue, on the neuro-chemical parameter of interest, in this case the molecular forms of β-endorphin. To address this, we conducted experiments with rats which mimicked the treatment conditions for human autopsy material. We stored the hypothalami from groups of eight rats at 4°C for 0, 2, 6, 12 or 24 hours and then measured total β-endorphin levels in each tissue. We then pooled the tissue extracts in each group and separated β-endorphin peptides by cation exchange chromatography. We found that storing hypothalmic tissue at 4°C for six hours or more significantly lowered the total β-endorphin content of the hypothalamus (Fig. 3). Nevertheless, cation exchange chromatography revealed that the β-endorphin processing

pathway was the same in tissues that had been maintained at 4°C for 2, 6, 12 or 24 hours as it was in tissues frozen immediately after the rats were killed (Fig. 4). The molecular forms of β-endorphin were similarly unaffected when brain tissue was stored for up to eight hours at room temperature (data not shown). These results indicate that while some β-endorphin is lost during the postmortem interval the overall processing pattern still reflects that present immediately after death.

Human brain samples were provided by the Brain Tissue Resource Center of McLean Hospital, an affiliate of Harvard Medical School. The subject population ranged in age between 62 and 81 years, all of whom died from cardiovascular disease (i.e., myocardial infarction, congestive heart failure) and none of whom were taking drugs known to affect brain β-endorphin. The postm.rtem interval was between two and seventeen hours. We have, thus far, exa.....d four samples; figure 5 illustrates a representative chromatogram. This shows that β-endorphin-1-31 is the predominant form of the peptide in the human hypothalamus; however, substantial amounts of N-acetylated and C-terminally shortened forms are also present. Of particular interest is the significantly greater proportion of acetylated β-endorphin peptides found in human brain compared to the rat (Fig. 4). Acetylated β-endorphin constituted approximately 35% of total immunoreactivity in the human hypothalamus but only 10% or less in the rat. We have also attempted to analyze the processing pattern of β-endorphin in CSF and in the amygdala, nucleus accumbens and subregions of the hypothalamus but these experiments were unsuccessful owing to the very low levels of β-endorphin present in these samples.

These studies are the first to identify the β-endorphin processing pathway in human brain. They provide evidence that the same, or similar, enzymes process β-endorphin in both the rat and human. Interestingly, peptide acetyltransferase appears to be a more critical determinant of the biological activity of β-endorphin in the human brain than in the rat. These results provide additional support for the concept that chemical agents designed to alter the activity of peptide acetyltransferase will have important effects on the physiology of brain β-endorphin and may be of significant clinical utility.

4. The isolation of peptide acetyltransferase.

a) **Preparation of an affinity label, bromoacetyl coenzyme A:** Affinity labeling is an effective and commonly used strategy for enzyme isolation. This approach utilizes an active-sight directed ligand which binds irreversibly to the enzyme to be isolated. By radiolabeling the affinity ligand, the enzyme itself can be effectively labeled. This has two distinct advantages for isolating the enzyme. First, it greatly facilitates the development of chromatographic and gel electrophoretic methods; the enzyme can be detected by following the peak of radioactivity rather than by the laborious and time consuming procedure of assaying the enzyme activity in each chromatographic fraction or gel band. Secondly, for enzymes which are unusually unstable, it provides an avenue for isolating the enzyme, at least for the purpose of developing antisera, which does not require maintaining the enzyme in an active, assayable form. One caveat, of course, is that the enzyme must still be clearly identified. This can be accomplished both by starting with a partially enriched enzyme preparation, isolated from other, similar enzymes which may also bind the affinity ligand, and by firmly identifying the enzyme according to its known properties once it is isolated.

Bromoacetyl coenzyme A is an irreversible inhibitor of choline acetyltransferase and a variety of other acetyltransferases (26) which binds covalently to the acetyl coenzyme A binding site common to all acetyl coenzyme A dependent enzymes. We reasoned that, because peptide acetyltransferase utilizes acetyl coenzyme A as a source of acetyl groups, bromoacetyl coenzyme A should bind irreversibly to the enzyme and function as an affinity label. Indeed, ^{14}C-labeled bromoacetyl coenzyme A has been used successfully as an affinity label for 5-hydroxy-5-methylglutaryl-coenzyme A synthase, also an acetyl coenzyme A dependent enzyme (27). Thus far, we have succeeded in developing methods for the synthesis and purification of bromoacetyl coenzyme A and, in preliminary experiments, have found that it inhibits peptide acetyltransferase activity in pituitary homogenates.

Bromoacetyl coenzyme A was synthesized by acetylating coenzyme A with bromoacetyl bromide according to the method of Lowe and Tubbs (27). The compound was purified and separated from residual coenzyme A by high performance liquid chromatography using an isocratic mobile phase consisting of 50 mM phosphate buffer (pH 5.3) in 15% methanol (28). Bromoacetyl coenzyme A eluted in a single peak with a retention time of fifteen minutes, clearly separated from coenzyme A (4 min) and acetyl coenzyme A (8 min). Bromoacetyl coenzyme A (100 uM) inhibited the peptide acetyltransferase activity of neurointermediate pituitary homogenates following a ten minute incubation at 37°C. These data demonstrate the feasibility of using the compound as an affinity label to facilitate isolation of the enzyme.

b) The source of acetyl groups for the acetylation of β-endorphin: Experiments were initiated during this project period to determine the source of acetyl groups used by peptide acetyltransferase to acetylate β-endorphin peptides. It has been assumed that the enzyme utilizes acetyl coenzyme A as a cofactor in vivo, as it does in in vitro enzyme assays although this had not been demonstrated. If so, we hypothesized that incubating intermediate lobe cells with ^{14}C-labeled glucose should label acetyl coenzyme A and, thereby, acetylated β-endorphin peptides, through the glycolytic pathway and the oxidation of pyruvate. To test this, we incubated primary cultures of rat intermediate lobe cells with ^{14}C-glucose (5 mM; 0.5 Ci/mmol) for three days and separated β-endorphin peptides by cation exchange chromatography. We found that ^{14}C-glucose did, in fact, label acetylated β-endorphin peptides; a major peak of radiolabel co-eluted with N-acetyl -β-endorphin-1-27 and small amounts of N-acetyl-β-endorphin-1-26 and -1-31 were also identified. No radiolabeled forms of non-acetylated β-endorphin were found, indicating that ^{14}C-glucose labeled the N-acetyl group and not the amino acids used to synthesize β-endorphin. These experiments are the first to demonstrate that acetyl coenzyme A serves as a cofactor for peptide acetyltransferase intracellularly. They have also successfully established a biosynthetic labeling paradigm which will, in future experiments, enable us to explore several important basic research questions regarding the mechanism used to transport acetyl coenzyme A into secretory vesicles. This experimental paradigm will also be readily applicable to the development of inhibitors of acetyl coenzyme A transport and of peptide acetyltransferase itself.

CONCLUSIONS

1. <u>Co-regulation of peptide acetyltransferase, POMC biosynthesis and β-endorphin processing in the intermediate pituitary.</u>

The results of these studies clearly establish the feasibility of selectively controlling the biosynthesis of β-endorphin peptides using chemical agents targeted on cell surface receptors to modify the activity of post-translational processing enzymes. For the endocrine POMC system, however, evaluating the significance of altered β-endorphin processing depends upon a definitive understanding of the physiologic function of the intermediate pituitary as well as the identification of the individual target sites of the multiple molecular forms of β-endorphin released from the intermediate lobe into the circulation. While the physiological function(s) of the intermediate pituitary are not completely understood, one of its most important roles appears to involve mediating the response of the organism to stressful stimuli. Previous studies from this and other laboratories (29, 30) have shown that stress, whether produced by surgery, pain, immobilization or changes in ambient temperature, is the most potent stimulus known for releasing β-endorphin from the intermediate lobe. Furthermore, stress releases β-endorphin both by activating neuronal pathways projecting from the hypothalamus and by elevating circulating epinephrine released by sympathetic activation of the adrenal medulla (30). Thus, the intermediate pituitary appears to function as a neuroendocrine transducer system, integrating both neural and autonomic inputs to produce a hormonal output, β-endorphin and other POMC-derived peptides, which transmit chemically encoded information regarding stressful stimuli to the periphery.

What then are the ultimate target sites of the β-endorphin peptides released from the intermediate pituitary? It is important to note that the quantitatively major forms of β-endorphin in the intermediate lobe have no affinity for opioid receptors. However, recent studies have identified several distinct classes of not only opioid, but also nonopioid β-endorphin receptors possessing varying specificities for the N-terminal (31), C-terminal (32, 33) or mid-region (34) sequences of the peptide, in peripheral tissues and on cellular elements of the immune system. Indeed, differing β-endorphin receptors in the immune system appear to discriminate among very small changes in the sequence of the peptide. It is conceivable, therefore, that β-endorphin peptides released from the intermediate lobe may play a role in mediating the immune response to stress. Communications between the central nervous system and elements of the immune system by means of neuroendocrine peptides is currently an area of intensive investigation.

In light of these findings, we hypothesize that selective alterations in β-endorphin processing function as a signal to the periphery that the organism is experiencing a persistent stressful stimuli. This hypothesis is supported by the results of studies by Akil et al. who found that chronic, but not acute, stress differentially alters the post-translational processing of β-endorphin peptides released from the intermediate pituitary, producing changes in processing and biosynthesis similar to those we observed following chronic treatment with dopaminergic antagonists (35). Their results clearly show that long term stress alters the molecular forms and, thus, the bioactivities of β-endorphin

peptides. The selective regulation of post-translational processing enzymes thus provides a mechanism for encoding information about the temporal nature of environmental stimuli perceived by the central nervous system and transmitted to the immune system and other peripheral tissues by the intermediate pituitary.

2. The regulation of β-endorphin processing in rat brain.

Neuronal β-endorphin processing is also modified by chemical agents which interact with cell surface receptors. However, the regulation of brain β-endorphin processing differs from that of the intermediate pituitary. Treatments which affect POMC gene expression in brain, such as gonadal steroids and chronic morphine administration, have no effect on the post-translational processing of β-endorphin whereas, in the intermediate lobe, changes in POMC biosynthesis are accompanied by specific alterations in the molecular forms of β-endorphin released from the gland. β-endorphin peptides are modified by dopaminergic agents although the specific changes which occur in brain differ from those in the pituitary. This indicates that additional, yet to be identified mechanisms regulate peptide processing in brain. Moreover, we have concluded from these studies that brain β-endorphin processing is relatively resistant to modification by chemical agents targeted on cell surface receptors. This emphasizes the need to develop pharmacologic strategies for directly inhibiting, or activating, the enzymes which post-translational process β-endorphin.

3. The post-translational processing of β-endorphin in human brain.

Studies of β-endorphin processing in human brain demonstrate that the pattern of β-endorphin peptides in the human differs from that of the rat in two important respects. First, the quantitatively predominant forms of the peptide in the human are N-acetylated and C-terminally shortened, opiate inactive forms of the peptide, whereas, in the rat, opiate active β-endorphin-1-31 is the primary product of the processing pathway. This changes our conceptualization of the 'endogenous opioid' β-endorphin neuronal system and emphasizes the importance of developing a more complete understanding of the physiological role subserved by non-opiate forms of the peptide in brain. Secondly, these studies indicate that peptide acetyltransferase is a critical determinant of the biological activity of β-endorphin peptides in human brain, unlike the rat, in which acetylated β-endorphin peptides are very minor products of the neuronal β-endorphin processing pathway. This indicates that inhibitors of peptide acetyltransferase may have important effects on the physiologic activity spectrum of β-endorphin and may of significant clinical importance.

FUTURE DIRECTIONS

The primary goal of future studies will be to purify and characterize peptide acetyltransferase. Our first approach to accomplishing this objective will be to usethe affinity label, bromoacetyl coenzyme A, to radiolabel the enzyme. Our initial efforts to synthesize and purify bromoacetyl coenzyme A have been highly successful. The next step will be to radiolabel the compound, using a slightly modified synthetic procedure, and to determine whether peptide

acetyltransferase can be effectively labeled by incubating the enzyme, partially purified from bovine intermediate lobe secretory vesicles, with ^{14}C-bromoacetyl coenzyme A. The availability of this affinity label will be a valuable asset for purifying peptide acetyltransferase. The first experiments will evaluate gel chromatography both as a purification step and for estimating the molecular size of peptide acetyltransferase. Gel electrophoresis will be used to monitor the enzyme purification procedure and as a second method for determining its molecular size. Subsequent experiments will focus on affinity chromatography as a primary approach to the purification of peptide acetyltransferase.

Two additional, ongoing projects will also continue into the ensuing project period. First, we plan to complete studies of the β-endorphin processing pathway in human brain. This requires analyzing a larger number of samples to ensure that our initial results are truly representative. We also plan to continue studies of the effects of the differing molecular forms of β-endorphin present in brain on both analgesia and cardiovascular function. The initial results of these experiments indicate that the processing of β-endorphin subserves very different roles in defining the hemodynamic and antinociceptive responses produced by β-endorphin peptides. These studies will enable us to more fully predict the effects of chemical agents targeted on post-translational processing enzymes.

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PUBLICATIONS

February 1, 1987 - January 31, 1988

Manuscripts Published During the Fiscal Year:

Chronwall, B.M., Millington, W.R., Griffin, W.S.T., Unnerstall, J.R. and
    O'Donohue, T.L. Histological evaluation of the dopaminergic regulation
    of proopiomelancortin gene expression in the intermediate lobe of the
    rat pituitary, involving in situ hybridization and (^{3}H)-thymidine uptake
    measurement. Endocrinology 120: 1201-1211, 1987.

Millington, W.R., O'Donohue, T.L. and Mueller, G.P. Dopaminergic agents
    selectively alter the post-translational processing of β-endorphin in
    the intermediate pituitary of the rat. Journal of Pharmacology and
    Experimental Therapeutics 243: 160-170, 1987.

Manuscripts Submitted for Publication During the Fiscal Year:

Chronwall, B.M., Hook, G.R. and Millington, W.R. Dopaminergic regulation
    of the biosynthetic activity of individual melanotrophs in the rat
    pituitary intermediate lobe: A morphometric analysis by light and
    electron microscopy and in situ hybridization. Submitted to Endocrinology.

Berglund, L.A., Millington, W.R. and Simpkins, J.W. Gonadal steroid and
    chronic morphine treatment does not change brain post-translational
    processing of β-endorphin. Submitted to Life Sciences.

Manuscripts in Preparation:

Millington, W.R. and Chronwall, B.M. Dopaminergic regulation of the inter-
    mediate pituitary. Invited chapter to be published in Neuroendocrine
    Perspectives, Vol. 7, 1988.

Millington, W.R., Dybdal, N.O., Gehlert, D.R. and Mueller, G.P. Alterations
    in the post-translational processing, but not the biosynthesis, of β-end-
    orphin in equine Cushing's syndrome. To be submitted to Endocrinology.

Dybdal, N.O., Millington, W.R., Chronwall, B.M. and Mueller, G.P. β-endorphin
    peptides are acetylated in the equine anterior pitutiary. To be submitted
    to the Journal of General and Comparative Endocrinology.

Gehlert, D.R., Rinaudo, M.S., Bishop, J., Millington, W.R. and Chronwall, B.M.
    GABA-ergic regulation of pro-opiomelanocortin gene expression. To be
    submitted to Peptides.

Lavigne, G., Millington, W.R. and Mueller, G.P. Morphine analgesia is poten-
    tiated by cholecystokinin antagonists. To be submitted to Pain.

ABSTRACTS

February 1, 1987 - January 31, 1988

Abstracts Presented During the Fiscal Year:

Chronwall, B.M., Millington, W.R., Hook, G.R. and O'Donohue, T.L. Histological evaluation of the dopaminergic regulation of POMC gene expression in the intermediate lobe of the rat pituitary. Xth International Congress of Pharmacology, 1987.

Gehlert, D.R., Dybdal, N.O., Millington, W.R. and Chronwall, B.M. Cushing's syndrome in the horse: Pituitary histology and evaluation of POMC gene expression. Society for Neuroscience, 1987.

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Berglund, L.A., Millington, W.R., Manzini, C., Mueller, G.P. and Simpkins, J.W. Gonadal steroid treatment does not change brain post-translational processing of β-endorphin. Society for Neuroscience, 1987.

Millington, W.R. Differential regulation of β-endorphin processing in the intermediate pituitary. Winter Conference on Brain Research, 1988.

Abstracts Submitted During the Fiscal Year:

Lavigne, G., Millington, W.R., Dionne, R.A. and Mueller, G.P. The interaction between cholecystokinin and opioid systems in the reaction to thermal stimuli. International Association for Dental Research, 1988.

Hirsch, M.D., Millington, W.R., McKenzie, J.E. and Mueller, G.P. Carboxy-terminal proteolysis of β-endorphin-1-31 to β-endorphin-1-27 enhances hypotensive potency. Endocrine Society, 1988.

Table 1. Intermediate lobe dopamine, DOPAC, serotonin and 5-HIAA levels in equine Cushing's syndrome.

	Dopamine (pmol/mg prot)	DOPAC (pmol/mg prot)	Serotonin (pmol/mg prot)	5-HIAA (pmol/mg prot)
Control	23.2 ± 3.8	8.0 ± 1.8	11.0 ± 1.9	2.5 ± 0.5
Cushing's	2.7 ± 3.8**	ND	15.3 ± 5.6	3.0 ± 0.8

Dopamine, DOPAC (3,4-dihydroxyphenylacetic acid), serotonin and 5-HIAA 5-hydroxy-indoleacetic acid) were analyzed by high performance liquid chromatography with electrochemical detection (15). The data are expressed as mean ± SEM and were analyzed by two-tailed Student's t test. N = 5-10 animals in each group. ND = not detectable. ** P<0.01 differs from control

Table 2. Plasma and CSF β-endorphin levels in equine Cushing's syndrome.

	Plasma β-endorphin (fmol/ml)	CSF β-endorphin (fmol/ml)
Control	38.44 ± 4.59 (21)	9.06 ± 0.47 (18)
Cushing's	2455.54 ± 651.6 (12)**	975.58 ± 314.16 (9)**

Plasma β-endorphin was analyzed by radioimmunoassay in 200 μl of unextracted plasma (6). CSF levels were assayed by lyophilizing 5 ml CSF, reconstituting in in 1.0 ml of water and assaying 200 μl aliquots of the reconstituted sample. The number in parentheses indicates the number of subjects in each group. The data are presented as the mean ± SEM and were analyzed by two tailed Student's t test. ** P<0.01 differs from control.

Table 3. Intermediate lobe β-endorphin and POMC mRNA levels in equine Cushing's.

| | β-endorphin (nmol/mg protein) | POMC mRNA | |
		Dot-Blot Assay (optical density)	In Situ Hybridization (nCi/mg tissue)
Control	95.98 ± 10.80 (17)	164.2 ± 10.0 (7)	9.80 ± 1.92 (4)
Cushing's	92.21 ± 12.75 (12)	186.6 ± 12.7 (5)	9.61 ± 1.51 (4)

β-endorphin levels were analyzed by radioimmunoassay (6) and POMC mRNA was determined by both dot-blot and in situ hybridization assays (6,13, Appendix I). The data from dot-blot hybridization assays are expressed as the optical density of autoradiographic film exposed to nitrocellulose filters containing the hybridized samples. For in situ hybridization experiments autoradiographic grain counts overlying tissue sections were converted to nCi ^{35}S-POMC cDNA/mg tissue using tissue standards included with each assay. The data are expressed as mean ± SEM and were analyzed by Student's t test.

Table 4. Intermediate lobe β-endorphin peptides in equine Cushing's syndrome.

| | β-Endorphin (percent eluted) | | | | |
	Ac-β-End-1-26	Ac-β-End-1-27	β-End-1-27	Ac-β-End-1-31	β-End-1-31
Control (11)	9.2 ± 1.1	57.3 ± 2.4	8.2 ± 2.5	12.8 ± 2.3	6.7 ± 0.8
Cushing's (8)	5.2 ± 0.5**	36.8 ± 2.4**	11.1 ± 2.0	20.9 ± 1.9*	22.1 ± 3.3'

β-endorphin peptides were separated by cation exchange chromatography (11) from extract of individual intermediate lobes. The data are expressed as the mean ± SEM of the indicated number of animals (n) and were analyzed by analysis of variance followed by Duncan's multiple range test. * P<0.05 and ** P<0.01 differs from control. β-End = β-endorphin and Ac = acetyl.
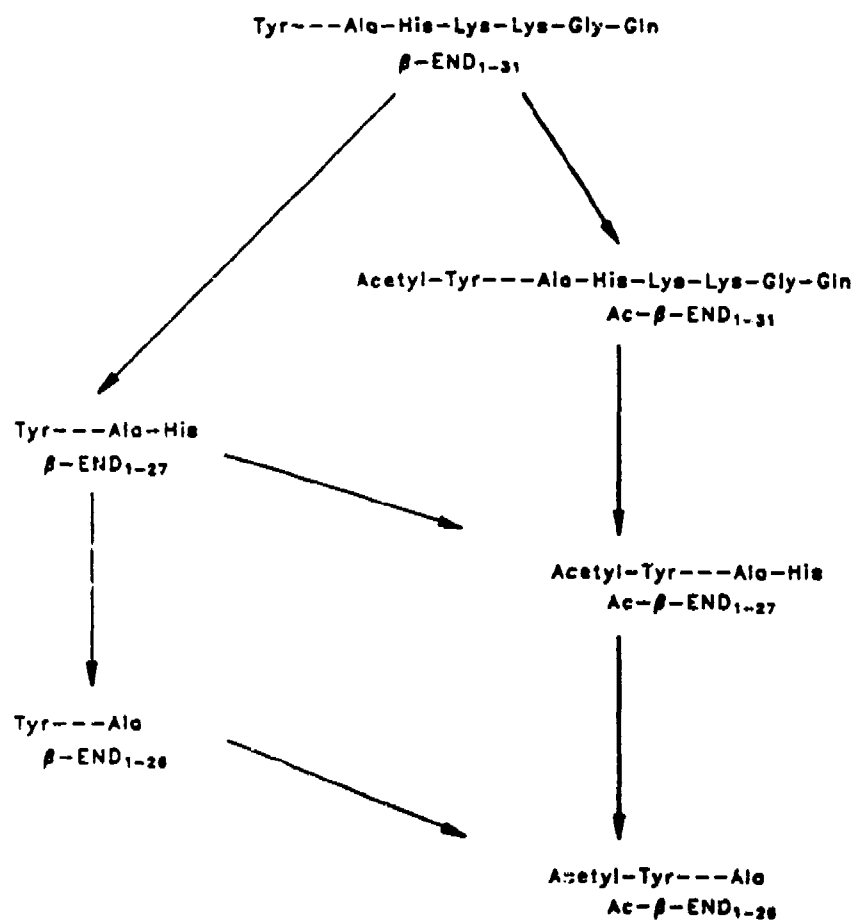
Figure 1. The post-translational processing of β-endorphin in the intermediate pituitary of the rat. Adapted from reference 7.
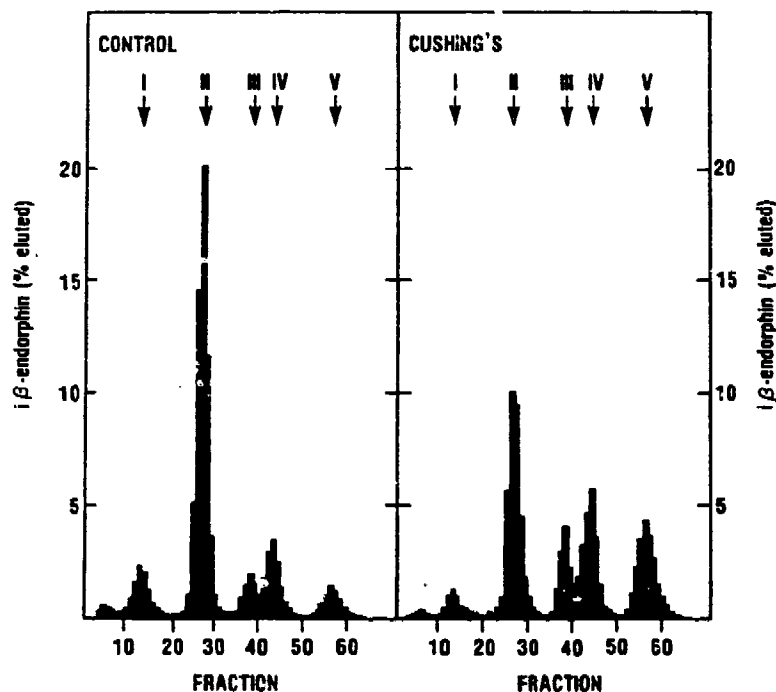
FIGURE 2

Figure 2. Intermediate lobe β-endorphin peptides in equine Cushing's syndrome. The intermediate lobes from eleven control and eight Cushing's horses were homogenized in 1 N acetic acid, aliquots of the pituitary extracts were pooled and β-endorphin peptides were separated by cation exchange chromatography using an SF-Sephadex column (1.5 x 15 cm) (11). The peaks of β-endorphin immunoreactivity are (I) N-acetyl-β-endorphin-1-26; (II) N-acetyl-β-endorphin-1-27; (III) β-endorphin-1-27; (IV) N-acetyl-β-endorphin-1-31; (V) β-endorphin-1-31.
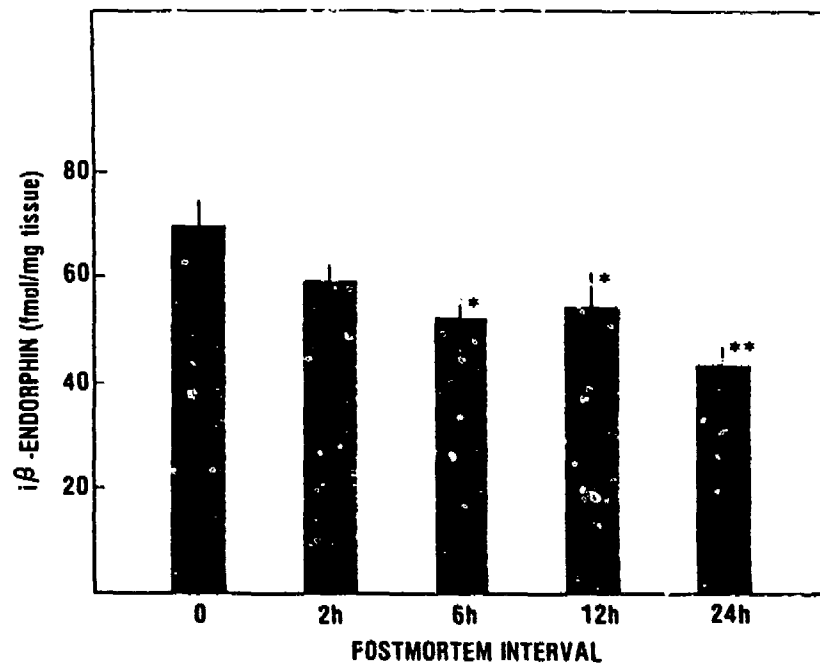
FIGURE 3

Figure 3. The postmortem decline in β-endorphin levels in rat hypothalamus. Groups of eight rats were killed by decapitation and the hypothalamus from each animal was removed and stored at 4° C for the indicated time interval. The tissues were then homogenized in 1 N acetic acid and β-endorphin levels were determined by radioimmunoassay. The data are presented as the mean ± SEM and were analyzed by analysis of variance followed by Duncan's multiple range test. * P<0.05 and ** P<0.01 differs from control.
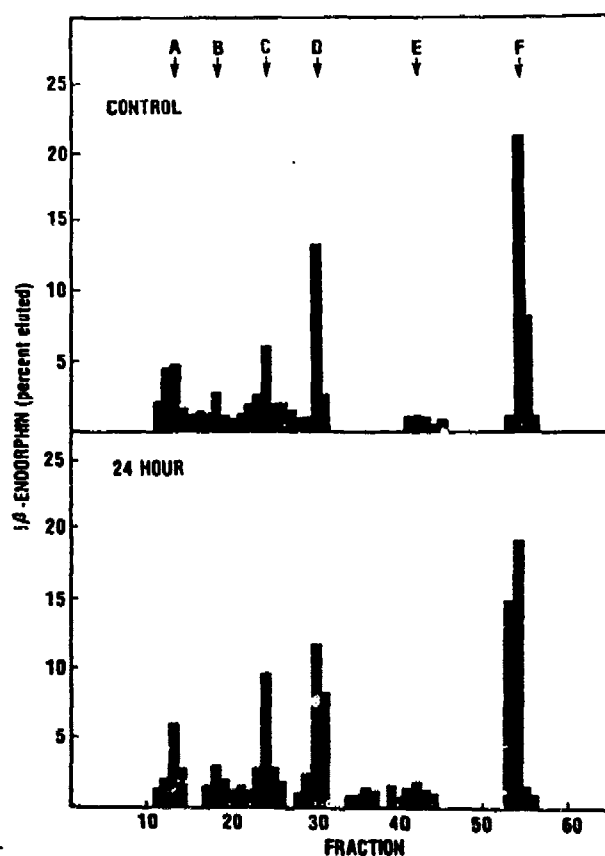
FIGURE 4

Figure 4.   Post-mortem storage does not affect the post-translational processing
of β-endorphin in rat hypothalamus.   The hypothalami from eight rats were either
frozen immediately after the animals were killed or stored at 4° C for twenty-
four hours.   The tissues were then homogenized in 1 N acetic acid and aliquots
of the tissue extracts were pooled and β-endorphin peptides were separated by
cation exchange high performance liquid chromatography.   The arrows mark the
elution positions of (A) N-acetyl-β-endorphin-1-26; (B) N-acetyl-β-endorphin-
1-2/; (C) β-endorphin-1-26; (D) β-endorphin-1-2/; (E) N-acetyl-β-endorphin-1-31;
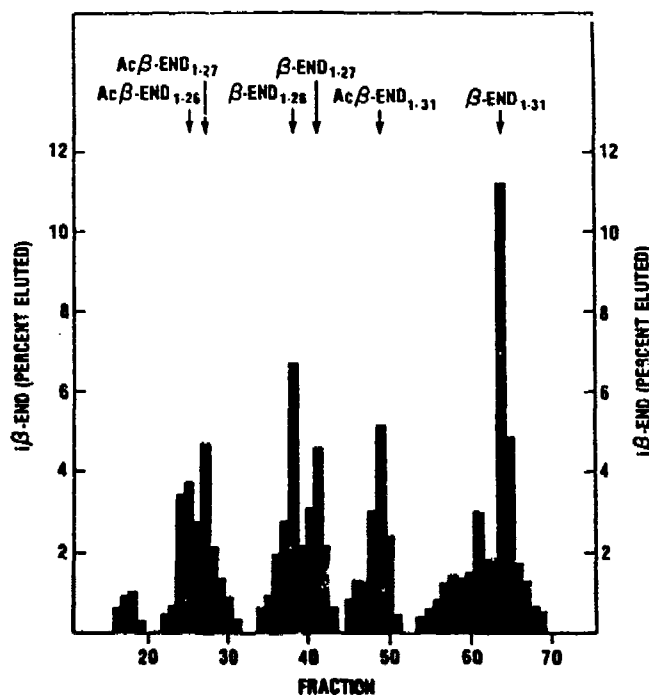(F) β-endorphin-1-31.

FIGURE 5

Figure 5. The post-translational processing of β-endorphin in human hypothalamus. The hypothalamus from a sixty-one year old male was homogenized in 1 N acetic acid and β-endorphin peptides were separated by cation exchange high performance liquid chromatography (11). The postmortem interval was three hours.